Contribution of acetic acid to the hydrolysis of lignocellulosic biomass under abiotic conditions

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Abstract

Acetic acid was used in abiotic experiments to adjust the solution pH and investigate its influence on the chemical hydrolysis of the Organic Fraction of Municipal Solid Waste (OFMSW). Soluble chemical oxygen demand (SCOD) was used to measure the hydrolysis under oxidative conditions (positive oxidation-reduction potential values), and pH 4 allowed for 20% (±2%) of the COD added to be solubilized, whereas only 12% (±1%) was solubilized at pH7. Under reducing conditions (negative oxidation-reduction potential values) and pH 4, 32.3% (±3%) of the OFMSW was solubilised which shows that acidogenesis at pH 4 during the anaerobic digestion of solid waste can result in chemical hydrolysis. In comparison, bacterial hydrolysis resulted in 54% (±6%) solubilization.

Keywords : Organic Fraction of Municipal Solid Waste, lignocellulosic biomass, pre-treatment, chemical hydrolysis.

1. Introduction

Lignocellulosic substrate is a general term for material containing cellulose, hemicellulose and lignin, and includes the Organic Fraction of Municipal Solid Waste (OFMSW), wood, grass, leaves, paper, wheat straw, etc. Cellulose is a linear polymer of glucose units which can form intra- and interchain bonds leading to a crystalline macromolecule. It is relatively rigid with a high degree of dimensional stability in the direction of the cellulose fibres. Hemicelluloses have a more irregular structure with side groups, substituent groups and sugars present along the length of the chain. Lignin is a

randomised condensed polymer with many aromatic groups and is much more hydrophobic than cellulose or hemicelluloses (Popescu et al., 2011).

Most of the cellulose is located in highly ordered crystalline regions, in which cellulose chains or fibrils are so tightly packed that even water molecules can scarcely penetrate; cellulose is accordingly water insoluble. Less ordered portions of the assembly, called amorphous regions, typically comprise about 5 percent of the cellulose microstructure (Bailey & Ollis, 1986). These amorphous regions are easily hydrolysed by, for example, acids; the crystalline regions on the other hand are much more difficult to decompose and it depends on crystallinity, crystallite size, degree of polymerisation, surface area, particle size, lignin content, wood density, etc (Hendriks & Zeeman, 2009; Popescu et al., 2011). The solubilization of lignocellulosic components not only depends on temperature, but also on other parameters like moisture content and pH. The xylan of hemicellulose can be extracted quite well in an acid or alkaline environment, while glucomannan is difficult to extract in an acid environment and needs a stronger alkaline environment than xylan to be extracted (Fengel & Wegener, 1984).

However, much more recalcitrant is the lignin casing which encloses the polysaccharide components of the biomass. Lignin is a complex and heterogeneous material, and its random arrangement makes it very resistant to chemical and enzymatic attack. The lignocellulosic complex prevents enzymes from accessing the degradable cellulose, and some studies have suggested that enzymes may adsorb preferentially onto lignin which results in unproductive enzyme binding (Kristensen et al., 2007). As both accessibility of enzymes to the solid matter and hydrolysis of complex compounds constitute the rate-

limiting step during anaerobic degradation (Eastman & Ferguson, 1981), pre-treatment of the substrate is beneficial for the rate and extent of the anaerobic process, the biogas yield and mass reduction of anaerobic sludge (Xiao & Clarkson, 1997).

Acid hydrolysis of cellulose is a well-known phenomenon and is carried out with concentrated or dilute acid: the most extensively used acids are H_2SO_4 and HCl (Grethlein, 1984). Rajan and Carrier (2014) determined the optimal conditions for pretreating wheat straw, and found them to be: 140°C, 1% (v/v) H_2SO_4 and a 30 min reaction time. Under these conditions, the glucose yield from wheat straw was maximized at 89% of the theoretical maximum. However, the use of acid is a deterrent to large scale implementation due to the cost of recovery of the expensive acid (Grethlein, 1984). Xiao and Clarkson (1997) treated newsprint paper with acetic acid alone at various concentrations (from 0 to 80%) in a boiling water bath, but no significant solubilization of lignin occurred. They showed that the addition of nitric acid had a tremendous effect on the solubilization of lignin. More recently it has been reported that the presence of lignin can slow down the acid hydrolysis of cellulose (Yoon et al., 2014). Acid hydrolysis of woody biomass was firstly carried out at high concentration (72% H_2SO_4 for 1 hr at 30°C) followed by a dilute acid hydrolysis (4% at 100-120°C), and it was shown that the second hydrolysis at 120°C could degrade glucose to 5-hydroxymethylfurfural (5-HMF) and then to levulinic and formic acids. However, the presence of lignin prevented the formation of levulinic acid and formic acid as part of the glucose or 5-HMF were converted to humins under acidic conditions (Yoon et al., 2014). Acids such as acetic acid were used as catalysts for the hydrolysis of lignocellulosics

in ionic liquids as this can take place at milder conditions. Van Spronsen et al. (2011) showed that acetic acid also works as co-solvent, increasing the solubility of the constituents of lignocellulosic biomass in the ionic liquid. No paper has yet examined the relative contribution of acetic acid and enzymes in the hydrolysis process of OFMSW. Sometimes, an imbalance in anaerobic digestion leads to an accumulation of acids and it is not clear at what concentration these acids can lead naturally to a chemical hydrolysis and to which extent. Acetic acid is the most common acid produced by acidogenic bacteria, and a two-stage anaerobic process allows for the optimization of the acid-phase reactor independently from the methanogenic reactor. Therefore, simultaneous chemical and bacterial hydrolysis could take place in the acidogenic reactor, but the contribution of each treatment on the overall solubilization is not known. Thus, the aim of this work was to investigate the effect of acetic acid concentration on chemical hydrolysis, and compare it with bacterial hydrolysis.

2. Materials and methods

2.1. Feedstock

The Organic Fraction of Municipal Solid Waste (OFMSW) was used in this study to simulate a lignocellulosic material that undergoes anaerobic digestion. It consisted of 41.3% Kitchen Wastes (KW), 10.8% Garden Wastes (GW) and 47.9% Paper Wastes (PW) on a wet basis. The composition of the simulated paper waste and the preparation of the feedstock used for the study was reported elsewhere (Trzcinski & Stuckey, 2009). This slurry was autoclaved (120°C for 20 minutes) to sterilize the feed.

2.2. Bacterial hydrolysis

To study the hydrolysis of OFMSW due to enzymes secreted by bacteria, an anaerobic inoculum (TSS = 2.7 g/L, VSS = 2.07 g/L) from a CSTR fed on the same substrate at 10 days HRT was added to a serum bottle with two grams of MSW so that an inoculum to substrate ratio (I/S) of 1.2 was obtained. The inoculum was previously centrifuged at 3,000 rpm for 20 minutes in order to discard the debris present in the supernatant. In order to study only the hydrolysis, methanogenesis was inhibited by adding Sodium Bromoethanesulfonate (BES) so that a final concentration of 10^{-5} M was obtained (Smith, 1983). A positive control was run in parallel with α-cellulose (Sigma-Aldrich) (on the same COD basis as OFMSW assuming that α-cellulose was $C_6H_{10}O_5$) to confirm the enzymatic activity.

The bottles were then filled to 100 mL with biomedium defined by Owen *et al.* (1979) and flushed with a CO_2/N_2 mixture (30/70%) for 5 minutes to obtain anaerobic conditions. A negative control with inoculum, biomedium and BES (no OFMSW) was run as well and its SCOD was subtracted from the bottle with OFMSW in order to obtain a SCOD which reflects enzymatic hydrolysis. The bottles were stoppered with rubber septum-type stoppers which were secured with 20 mm aluminium crimp seals, and placed on an orbital shaker at 30°C. All bottles were run in triplicate, and the values reported are the mean of the 3 values.

2.3. Chemical hydrolysis under oxidative conditions

To study the influence of pH of the medium on chemical hydrolysis, pHs of 4, 5, 6, 7 were tested using acetic acid which is the most common volatile fatty acids measured during the anaerobic digestion process. Abiotic serum bottles of 120 mL were used as batch reactors at 30°C; a bottle without pH control, and a bottle at pH 9 (using NaOH) were run in parallel. Two grams of OFMSW slurry were added to each bottle and tap water was added to reach a final volume of 100mL. In order to study the effect of pH only, bacterial growth was inhibited by adding $K_2Cr_2O_7$ so that a final concentration of 100 mg Cr/L was obtained. Such a high chromium level was deemed lethal according to Aquino and Stuckey (2004). The pH was then carefully adjusted to 4, 5, 6, 7 by adding drops of dilute acetic acid. All bottles were run in triplicate, and the values reported are the mean of the 3 values. Samples were taken after 2, 5, 10, 15 and 25 days to analyze for SCOD and acetic acid concentrations. The value of acetic acid was converted to COD by multiplying its concentration by 1.07 (Aquino & Stuckey, 2004) and then subtracting it from the Soluble COD concentration measured in order to obtain the actual SCOD concentration that reflects the chemical hydrolysis.

2.4. Chemical hydrolysis under reducing conditions

A similar experiment was carried out under reducing conditions, i.e. negative oxidation-reduction (redox) potentials which are commonly found in anaerobic environments. This time bottles were adjusted to pHs of 4, 5, 6, 7 with acetic acid and pH 9 with NaOH, and the redox potential was adjusted to between -100 and -200 mV by means of a diluted $Na_2S.9H_2O$ solution. Potassium dichromate was also added to avoid bacterial growth. These bottles

were regularly adjusted over three days to reach the required pH/redox equilibrium, and then OFMSW was added to start the experiment.

2.5. Analytical and statistical methods

The measurement of pH (Jenway 3020 pH Meter) was accurate to within ±0.02 units. The Soluble Chemical Oxygen Demand (SCOD) analysis was carried out as in Standard Methods (APHA, 1999) after filtration through a 0.45 µm filter (Sartorius, Minisart). The relative standard deviation for ten identical samples was 2.6%. The solubilization yield (%) was calculated as the SCOD in the supernatant divided by the total COD fed in the bottle. Acetic acid concentration was analyzed as previously described (Trzcinski & Stuckey, 2011). The oxidation-reduction potential was measured with a platinum-band ORP electrode (Cole-Palmer, USA) connected to a Jenway 3020 pH meter. The data were statistically analyzed using Excel Analysis Toolpak. Firstly, an *F*-Test was run to determine whether two sample populations had significantly different variances, or not, at a confidence level $\alpha = 0.05$. Secondly, a student's "*t*-test" was used to determine whether the mean values of two data columns were significantly different by testing the hypothesis that the means of the two columns are equal, assuming equal or unequal variances based on the *F*-test result. Means were reported as not significantly different, or significantly different, at a confidence level $\alpha = 0.05$. The surface area and pore width of the OFMSW were analyzed with a Micromeritics TRISTAR 3000 Analyzer.

3. Results and discussion

3.1. Bacterial hydrolysis

Bacterial hydrolysis proceeds by adsorption of exocellular enzymes onto the particulate substrate, together with their reaction with the soluble substrate (Noike et al., 1985), and also by attachment of the enzyme producing bacteria to the organic substrate particles (Vavilin et al., 1996). As Figure 1 shows, the SCOD concentration in the supernatant increased gradually due to the enzymatic activity of bacteria which converted the particulate OFMSW into soluble molecules. The absence of a lag phase suggests that the inoculum was well acclimatized to the substrate, and that some compounds in food, garden and paper wastes were readily hydrolysed by bacteria. After day 15, the curve reached a plateau meaning that the residual COD had stopped being hydrolysed. Alpha-cellulose had a lag phase for 2 days due to adaptation, and then reached 64% solubilization after 10 days showing satisfactory enzymatic activity of the inoculum. Typically OFMSW may contain up to 20-30% cellulose on a dry basis as well as ~5% lignin (Op den Camp et al., 1989), which limited the extent of solubilization compared to pure cellulose. A different crystallinity and the lignin casing may also explain the discrepancy with the positive control. The enzymatic hydrolysis of OFMSW achieved 54% solubilization and this was significantly greater than the chemical hydrolysis at all pH's tested at the 95% confidence level.

3.2. Chemical hydrolysis

In the bottle without pH control (dotted line in Figure 1) the SCOD increased slightly from 248 mg/L on day 2 to 284 mg/L on day 25, which shows that OFMSW did not solubilise readily under neutral conditions. The initial SCOD consisted of soluble compounds present in the OFMSW such as food waste

and some compounds possibly hydrolysed due to autoclaving the OFMSW slurry.

Table 1 shows the concentration of undissociated acetic acid in the abiotic bottles at each pH tested. It is clear that higher concentrations led to greater SCOD concentrations in the supernatant, and consequently a higher solubilization yield. This may due to the hydrolysis of acetyl groups on the hemicellulose and the depolymerization to galactose, glucose, and mannose from (galacto)glucomannan, and xylose from glucuronoxylan (Yoon et al., 2014). Previous work on the hydrolysis of straw and wood with ionic liquids at 398K showed that furfural production increased when the concentration of acetic acid increased (van Spronsen et al., 2011).The pH 4 bottle contained 1.4 g/L of undissociated acid, and about 20% (±2%) of the total COD fed to the bottle was solubilised which was significantly greater than 12% at pH7 (Table 1). It is important to note that at pH 4, 85% of the acid is in the undissociated form (CH_3COOH), while at pH7 this fraction is only 0.5%. It is clear from the results that these acidic and oxidative conditions resulted in the chemical hydrolysis of OFMSW.

In the second experiment (Figure 2) we attempted to study the influence of acidic conditions but under reduced environmental conditions commonly found in anaerobic digestion processes. Similarly to oxidative conditions, the results (see Figure 2) showed that there is a positive effect of acetic acid on the chemical hydrolysis of OFMSW. A 32.3% (±3%) solubilization of the OFMSW fed was achieved at pH4, whereas only 14.4% (±1%) occurred at pH7 (Table 1). As can be seen in Table 1, to reach pH 4 under reduced conditions, a high final concentration of acetic acid (~8 g/L) was required to

observe such significant chemical hydrolysis. At pHs in the range 5-7, the chemical hydrolysis of OFMSW by acetic acid can be considered negligible.

The surface area and the pore width of dried OFMSW samples after treatment are listed in Table 2. Interestingly, the enhanced solubilization did not correlate with surface area but with the pore width. This indicates that acetic acid can increase the pore width of OFMSW. Such an increase in pore size can be related to the removal of hemicellulose as reported earlier (Gregg & Saddler, 1996). An increase in micropore size due to the enzymatic hydrolysis of microcrystalline cellulose treated with rumen fluid was also observed by scanning electron microscopy (Song et al., 2005). Earlier work by Grethlein (1985) showed a linear correlation between the initial hydrolysis rate of pretreated biomass and the pore size accessible to a molecule with a diameter of 5.1 nm, which is about the diameter of a 'representative' cellulase. The observed increase in the mean pore size can, therefore, increase the probability of cellulose hydrolysis.

4. Conclusion

This work has shown that significant chemical hydrolysis by acetic acid can take place under oxidative and reduced environments. Under oxidative conditions at a concentration of 1.4 g/L acetic acid can lead to a 20% solubilization yield. Under reduced conditions at an acetic acid concentration of about 8 g/L and pH 4 a maximum solubilization yield of 32% was obtained, but this is still much lower than the enzymatic hydrolysis by bacteria which in comparison can achieve 54% COD solubilization. Surface area analysis

revealed that acetic acid chemically hydrolyzes OFMSW by increasing its pore width.

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